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Heinonen, Sini

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White adipose tissue mitochondrial metabolism in health and in obesity

Sini Heinonen[1] | Riikka Jokinen[1] | Aila Rissanen[1,2] | Kirsi H. Pietiläinen[1,3]

[1] Obesity Research Unit, Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki, Helsinki, Finland

[2] Department of Psychiatry, Helsinki University Hospital, Helsinki, Finland

[3] Endocrinology, Abdominal Center, Helsinki University Hospital, Helsinki, Finland

Correspondence
Kirsi H. Pietiläinen, MD, PhD, Obesity Research Unit, Biomedicum Helsinki, Helsinki University Central Hospital, C424b, PO Box 700, Haartmaninkatu 8, Helsinki 00029, Finland.
Email: kirsi.pietilainen@helsinki.fi

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Summary

White adipose tissue is one of the largest organs of the body. It plays a key role in whole-body energy status and metabolism; it not only stores excess energy but also secretes various hormones and metabolites to regulate body energy balance. Healthy adipose tissue capable of expanding is needed for metabolic well-being and to prevent accumulation of triglycerides to other organs. Mitochondria govern several important functions in the adipose tissue. We review the derangements of mitochondrial function in white adipose tissue in the obese state. Downregulation of mitochondrial function or biogenesis in the white adipose tissue is a central driver for obesity-associated metabolic diseases. Mitochondrial functions compromised in obesity include oxidative functions and renewal and enlargement of the adipose tissue through recruitment and differentiation of adipocyte progenitor cells. These changes adversely affect whole-body metabolic health. Dysfunction of the white adipose tissue mitochondria in obesity has long-term consequences for the metabolism of adipose tissue and the whole body. Understanding the pathways behind mitochondrial dysfunction may help reveal targets for pharmacological or nutritional interventions that enhance mitochondrial biogenesis or function in adipose tissue.

KEYWORDS

adipose tissue, mitochondria, obesity

1 | INTRODUCTION

Obesity is a global and rapidly increasing problem, tripled since 1975 by WHO 2018 standards in developed countries. Obesity is also extremely difficult to treat. A key defining feature of obesity is an adipose tissue dysfunction, which is considered to be a major contributor to the development of obesity-related metabolic problems,[1,2] such as metabolic syndrome, insulin resistance, hypertension, dyslipidaemia, and fatty liver. The underlying pathological mechanisms that impair adipose tissue function in obesity are incompletely understood, but in the light of recent scientific advances, it may be connected to insufficient storage capacity or impaired function of mitochondria, or both.

Mitochondria are the energy centres of adipocytes and are involved in many of their key metabolic functions including ATP production, fatty acid synthesis and oxidation, and the triglyceride balance of the cell. Although adipose tissue was long considered as an inert reservoir of fat with low abundance of mitochondria, adipose tissue and its active mitochondria have recently emerged as one of the central regulators influencing whole-body metabolism.[1,3,4] Impairments in adipocyte mitochondrial function are associated with metabolic diseases and the development of obesity-related disorders.[3-6] Better understanding on the dysfunction of adipose tissue mitochondria may yield insights on how the metabolic complications of obesity could be reversed. In this review, we concentrate on the metabolic processes in white adipose tissue that are regulated by mitochondria

and aim to highlight the functions of this organelle in current research on obesity and adipose tissue.

2 | ADIPOSE TISSUE

White adipose tissue is one of the largest organs of the body. Approximately 10% to 20% of total body weight in lean adults is white adipose tissue, but in individuals with obesity, the amount can increase[7] up to 40% to 70%. By harvesting excess lipids and glucose from the circulation, it protects other tissues from the pathological accumulation of triglycerides.[2,8] When this storage capacity is disrupted, lipids may spill over into ectopic sites like internal organs and vasculature resulting in low-grade inflammation, insulin resistance, and metabolic problems.[2,8,9] Intriguingly, both a total lack of adipose tissue in lipodystrophies and an unhealthy excess of adipose tissue in obesity lead to the same complications, including liver fat accumulation and insulin resistance.[2,8] Moreover, adipose tissue is an active endocrine organ that regulates many metabolic responses at the whole-body level through adipocytokines.[10] Changes in the main adipokines have been implicated in many obesity-related metabolic problems, such as type 2 diabetes, metabolic syndrome, and cardiovascular diseases.

Adipose tissue consists of adipocytes and a matrix, which includes collagen, blood and lymphatic vessels, and the stromal vascular fraction of adipose tissue with endothelial cells, smooth muscle cells, immune cells, adipocyte precursor cells (preadipocytes), and mesenchymal stem cells.[11,12] Approximately 75% of adipose tissue weight and 95% of an adipocyte consist of triglycerides. The main depots of white adipose tissue are subcutaneous (80% to 90% of body fat), visceral (10% of body fat), and ectopic (intrahepatic, intramuscular, and intrapancreatic) fat.[13,14] Different adipose depots have differences in capacity for adipocytokine secretion and cell type composition.[15,16]

In addition to white adipose tissue, also brown adipose tissue (BAT) and beige/brite adipose tissue (having mixed characteristics of both white and brown adipose cells) in humans exist.[17-19] BAT has a distinctive brown colour, which originates from the high iron and cytochrome content of the dense network of mitochondria and vasculature within the tissue.[20] In contrast to the large unilocular triglyceride droplets in white adipocytes, brown adipocytes are composed of small, multilocular lipid droplets. BAT is the site of nonshivering thermogenesis, where the brown adipocyte-specific protein, uncoupling protein-1 (UCP1), physiologically uncouples the respiratory chain to generate heat, and its mitochondria could thus "burn" away fat.[17] An extensive previous research shows that BAT function is impaired and its activity reduced in obesity.[21,22] Cold-induced BAT glucose uptake and stimulation of blood flow are reduced in individuals with obesity[23] as well as glucose uptake rates into BAT lower in both individuals with obesity and with type 2 diabetes.[24] Studies have also shown that animals with more BAT are more resistant to obesity and type 2 diabetes.[25,26] However, as the amount of BAT in human adults is very low, the clinical significance and contribution of BAT to energy expenditure are still debated.[27] There is active research, reviewed elsewhere, on the possibilities of inducing "browning" of white adipose tissue or BAT function to treat obesity and its metabolic outcomes.[27-29]

This review concentrates on the mitochondria of white adipose tissue.

3 | MITOCHONDRIA

Mitochondria are essential for key adipose tissue functions (Figure 1). Mitochondria produce energy in the form of ATP through oxidative phosphorylation (OXPHOS), generate substrates for cell metabolism (eg, de novo fatty acid synthesis), regulate lipid turnover, and control the generation of new adipocytes and adipokine secretion.[3,30]

Mitochondria are double-membrane organelles with an outer and an inner membrane and an intermembrane space. The inner membrane is folded into cristae and surrounds a mitochondrial matrix, where many chemical reactions of energy metabolism take place. Mitochondria possess their own genome, a circular mitochondrial DNA (mtDNA), which encodes 13 proteins critical for OXPHOS and two ribosomal and 22 transfer RNAs required for mitochondrial ribosomes and translation, respectively.[31,32] In addition, over one thousand mitochondrial proteins, including essential proteins of OXPHOS, mitochondrial translation, and other mitochondrial processes are encoded by nuclear DNA.[31,33,34]

3.1 | Mitochondrial oxidative energy metabolism

The main energy derivation pathways of the cell, including pyruvate oxidation, fatty acid β-oxidation, the tricarboxylic acid (TCA) cycle, and OXPHOS, occur in mitochondria (Figure 1).[35]

In aerobic energy production through OXPHOS, high-energy electrons (derived from substrate oxidation) are transferred through the electron transport chain in the inner mitochondrial membrane (complexes I-IV of the OXPHOS system). The electron transport is coupled with proton pumping at complexes I, III, and IV, generating an electrochemical potential difference across the inner membrane. The energy of the gradient is utilized by complex V (ATP synthase) to phosphorylate ADP to ATP.[36]

The TCA cycle is the final common oxidative pathway for all substrates (carbohydrates, fatty acids, and amino acids) and generates the high-energy electron carriers (NADH and $FADH_2$) that supply OXPHOS, energy compounds ATP and GTP, and metabolites needed as carbon skeletons for many biosynthetic processes of the cell, such as de novo fatty acid synthesis.

Pyruvate derived from glucose is transported to the mitochondria and oxidized in the matrix yielding acetyl-CoA. The pyruvate dehydrogenase complex catalyses the reaction and controls the amount of acetyl-CoA fed into the TCA cycle.

Free fatty acids (FFAs) are metabolized, esterified, or β-oxidized in adipocyte mitochondria. The long-chain fatty acids are transported from the cell cytosol into the mitochondrial matrix by carnitine palmitoyltransferases (CPTs; CPT1, CACT, and CPT2).[37] β-oxidation of the fatty acids produces acetyl-CoA, which enters the TCA cycle.

Catabolism of branched-chain amino acids (BCAA, ie, leucine, isoleucine, and valine) also occurs in mitochondria. Branched-chain amino acid aminotransferase (BCAT) forms α-ketoacids (BCKAs) from BCAAs via both cytosolic (BCATc, BCAT1) and mitochondrial (BCATm, BCAT2) isoenzymes.[38] The BCKAs are transported into mitochondria, where they are decarboxylated by the mitochondrial branched-chain α-ketoacid dehydrogenase (BCKD) complex. Finally, the products are used in the TCA cycle.

3.2 | Energy-status–dependent regulation of mitochondria

By changing the morphology, distribution, and mass of mitochondria, the cell adapts to different energetic and metabolic demands.[39] Mitochondria are remodelled by fusion and fission, and changes in their rate of biogenesis and distribution in the cell are frequent.[40,41] Studies on mitochondrial remodelling in adipose tissue are, however, sparse. Mitochondrial network fragmentation and fission appears to improve mitochondrial bioenergetics and make adipose tissue more insulin sensitive.[42] This is in contrast to skeletal muscle, where fission contributes to insulin resistance.[43]

The energy status of the cell is signalled through the NAD^+:NADH ratio, the AMP:ATP ratio, and acetyl-CoA levels,[44,45] which sense the signals of mitochondrial activity. AMP-activated protein kinase (AMPK) is activated when AMP levels are high. This induces oxidative phosphorylation and suppresses cell growth and proliferation.[45] NAD^+-dependent deacetylase sirtuin 1 (SIRT1) is activated when NAD^+ levels are high, and this upregulates mitochondrial mass, ATP generation, and nutrient oxidation. Both AMPK and SIRT1 activate the peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α). PGC-1α is one of the main inducers of mitochondrial oxidative metabolism, has a major role in mitochondrial biogenesis,[46] and interacts with many mitochondria-related transcription factors.[47] In energy excess, PGC-1α is acetylated and silenced. Caloric restriction leads to PGC-1α activation through SIRT1.[48] An activated PGC-1α induces oestrogen-related receptor α (ERRα) and GA-binding protein α (GABPα), which increase the function of the OXPHOS complexes including cytochrome c and ATP synthase.[49,50] PGC-1α enhances nuclear respiratory factor 1 (NRF-1), which is needed for the induction of mitochondrial biogenesis[51] and TFAM, which controls mtDNA stability and the transcription of mtDNA-encoded genes.[52] Transcription factor Forkhead box O 1, FOXO1, enhances adipogenesis[53] and controls adipocyte stress response.[54] Also, mitochondrial DNA methylation may be a control factor of mitochondria, although recent studies have challenged its existence altogether.[55,56]

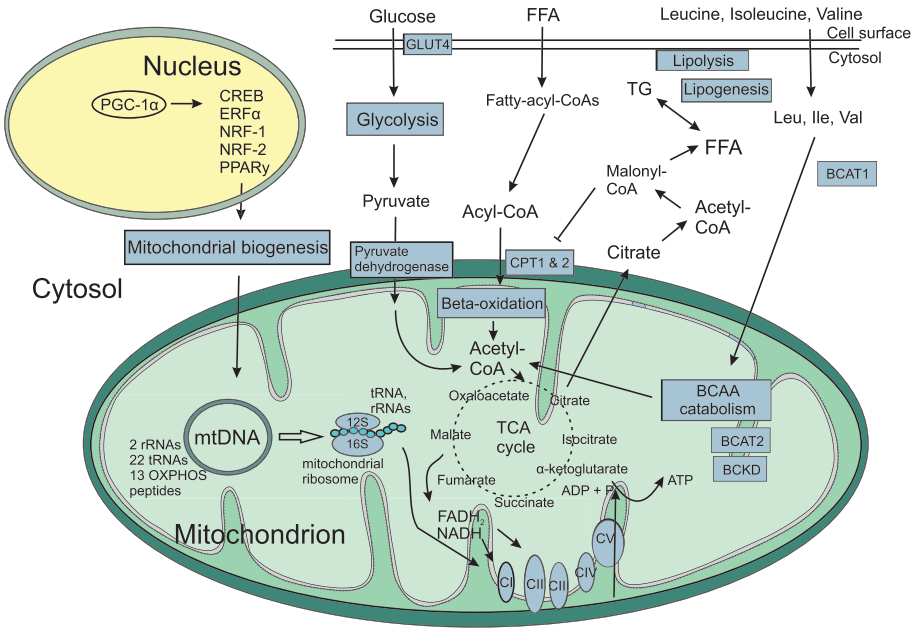

FIGURE 1 Normal mitochondrial function in adipose tissue. Mitochondria (in green) are regulated by various nuclear-related transcription factors. Most of the regulators are under the influence of PGC-1α (in nucleus, yellow background). In normal conditions, transcription factors enhance mitochondrial biogenesis and function. MtDNA (green circle) encodes proteins critical for mitochondrial ribosomes (12S and 16S subunits, in grey) and for the OXPHOS complexes (in the mitochondrial membrane, in grey). The translation of these proteins is processed in the mitochondrial ribosomes. Glucose, FFA, and BCAAs derived from nutrients (above the two lines, cell surface) are used for the energy production and other maintenance functions of the cell. Glucose is converted into pyruvate via glycolysis, and pyruvate-derived acetyl-CoA enters the TCA-cycle (dotted line, circle) for the production of ATP and GTP, NADH and $FADH_2$, as well as TCA-metabolites, like citrate. FFA-derived acyl-CoA enters beta-oxidation and acetyl-CoA further to TCA cycle. BCAAs are catabolized via BCAT1 in cytosol and BCAT2 in mitochondria. BCKD complex frees acetyl-CoA into the TCA cycle. Citrate is used for biosynthetic processes of the cell, like production of other TCA metabolites and as precursor for lipogenesis as malonyl-CoA, which also inhibits beta-oxidation through CPT1 transporters. Mark explanations: an arrow, induction; a T-line, inhibition; green area, mitochondrion; yellow area, cell nucleus

4 | MITOCHONDRIAL METABOLISM IN WHITE AT IN HEALTH AND IN OBESITY

4.1 | Mitochondrial oxidative metabolism in white AT is altered in obesity

In recent decades, altered mitochondrial oxidative metabolism has emerged as a molecular hallmark of obese adipose tissue (Figure 2).

Reduction of mitochondrial oxidative metabolism in adipose tissue in obesity[57,58] and in diabetes[59] has been demonstrated in several animal studies: In diet-induced or genetic mouse models of obesity, limited OXPHOS capacity, measured by maximal respiration capacity and cell respiratory control ratios via cell respirometer, was observed in white adipocytes, both in the absence and the presence of impaired glucose tolerance.[58] The authors concluded that impairments in mitochondria relate to obesity, not to glucose intolerance.[58] Mitochondria-related transcription was reduced, and mitochondrial staining, DNA quantification, and measurements of citrate synthase activity revealed reduced mitochondrial biogenesis in both obesity and diabetes.[60] The gene transcripts encoding mitochondrial proteins were decreased in obese mice without diabetes.[57] After treatment with rosiglitazone, half of the genes were upregulated, and the change was accompanied by an increase in mitochondrial mass.[57] However, another study found reduced levels of OXPHOS mitochondrial protein subunits, cellular mitochondrial DNA content by qPCR, oxygen consumption by cell respirometer, and number of mitochondria by MitoTracker staining and electron microscopy in diabetic, but not obese mice.[59]

Also, several human studies have linked obesity to mitochondrial dysfunction and to impaired glucose and lipid metabolism in adipose tissue.[3,4,30] We have previously shown that mtDNA amount and gene expression levels of mitochondria-related pathways are downregulated in co-twins with obesity compared with their co-twins who are lean, a rare study setting that distinguishes the acquired features of obesity from potential genetic effects.[61] Moreover, we have demonstrated downregulation of mitochondrial biogenesis in these twins with obesity compared with their lean identical co-twins by reduced expression of genes encoding for mitochondrial proteins, expression of PGC-1α, mtDNA amount, expression levels of mtDNA-encoded transcripts and mitochondrial ribosomal protein subunits, and, finally, reduced levels of OXPHOS complex subunits.[62] The reduced expression of nuclear-encoded mitochondria-related genes, PGC-1α, and reduction of mtDNA-encoded transcripts were recapitulated in isolated primary mature adipocytes of the identical twins and the reduced levels of OXPHOS protein subunit levels in the adipocytes of unrelated individuals with obesity versus lean individuals.[63] In within-pair extensive genome-wide DNA methylation analysis, we have previously identified 17 differentially methylated obesity-associated genes.[64] These genes clustered to downregulated lipid metabolism by mitochondria, downregulated adipogenic genes with upregulated inflammation, and extracellular matrix (ECM) remodelling. Additionally, we have shown hypermethylation in the co-twins with obesity in two CpG sites within the gene body of PGC-1α, with methylation in one of the CpG sites correlating with PGC-1α expression.[62]

imilar results have been obtained in studies of unrelated individuals. Downregulation of mitochondrial mtDNA in individuals with obesity has been shown by several studies,[61,62,65-69] although not all.[70-72] The expression of PGC-1α[73] and the activities of the OXPHOS complexes I to IV, mitochondrial phosphate utilization, and mitochondrial membrane potential[74] were downregulated in subcutaneous adipose tissue of patients with obesity, compared with lean controls. The activities of the OXPHOS complexes were reduced in simple obesity and in obesity with diabetes.[74] Obesity also links to decreased levels of OXPHOS complexes I and IV[75] in adipocytes, decreased mitochondrial oxygen consumption rates in isolated adipocyte mitochondria,[71,75] and reduced oxygen consumption rates in preadipocytes after beta-adrenergic stimulation.[71] A study with human preadipocytes has revealed changes in the methylation pattern of the preadipocytes obtained from individuals with obesity, with loss of DNA methylation in selected regions, where adipogenesis, inflammation, and immunosuppression were the most affected pathways.[76]

Proteomic studies revealed that BMI was inversely associated with four important omental adipose tissue mitochondrial proteins—citrate synthase, HADHA, LETM1, and mitofilin.[77] A lower abundance of mitochondrial proteins in subcutaneous adipose tissue has been recorded in insulin resistance without the presence of obesity[78] and in visceral fat of individuals with type 2 diabetes.[79] These studies suggest changes in the mitochondrial proteome with metabolic disorders in general, but exact studies on obesity have yet to be performed.

Primary mitochondrial defects in adipose tissue affect metabolic health also in transgenic mice. The results here, however, seem more pronounced and somewhat contradictory than in acquired obesity. ATP depletion by knocking out the TCA enzyme fumarase hydrogenase in white and brown adipocytes resulted in low adipose mass, small adipocytes, and protection against obesity, insulin resistance, and fatty liver despite a high-fat diet.[80] Mice genetically overexpressing prohibitin (needed in adipocyte differentiation) had enhanced mitochondrial biogenesis and consequently developed obesity.[69] These studies suggest that mitochondrial downregulation in adipose tissue could be beneficial, raising the question if the downregulation of mitochondria in obesity is a compensatory mechanism. However, this may not be the case as in another study with an adipose-specific TFAM-knockout mice model to simulate mitochondrial dysfunction, the mice became lipodystrophic and developed mitochondrial dysfunction, fatty liver, insulin resistance, and hypertension.[81] Nevertheless, the conditions in these primary genetic-model studies are not directly comparable with obesity in humans and mice, where mitochondrial downregulation relates to obesity and metabolic problems. The mice in the studies had primary mitochondrial defects, while the decrease of mitochondrial function occurring in obesity is milder, possibly involving many different aspects of mitochondrial function.

4.2 | Fatty acid oxidation is impaired in obesity

Adipocytes both generate and oxidize lipids. During nutrient excess, glucose metabolism and lipogenesis produce malonyl-CoA, which

inhibits the import of fatty acids to mitochondria through the CPT1 transporter, decreasing fatty acid oxidation. In low energy status, AMPK activation decreases malonyl-CoA,[82] resulting in enhanced CPT1 activity and β-oxidation. Sirtuins regulate AMPK and have lately emerged as important modulators of lipid metabolism and fatty acid oxidation.[83]

In obesity, mitochondrial fatty acid oxidation is suggested to be impaired,[4] at least in subcutaneous adipose tissue.[84] Obese rats have impaired fat oxidation and reduced CPT1 mRNA levels in intraabdominal adipose tissue.[85] CPT1A overexpression in cultured adipocytes in turn has enhanced fatty acid oxidation, improved insulin sensitivity, and decreased inflammation.[86] However, as only modest activity of CPT1 in adipose tissue has been suggested, it has been questioned whether the changes in the levels of CPT1 have significance in obesity.[87] On the other hand, in mice study, an adipose-specific knockout of CPT2A of mitochondrial long-chain fatty acid β-oxidation compromised fatty acid oxidation in adipose tissue but reduced high-fat diet-induced oxidative stress, ROS production, and inflammation compared with normal mice.[88] These results may indicate that an intricate balance of fatty acid oxidation may be needed—enough to prevent excess accumulation of triglycerides in adipose tissue and FFA flux to other tissues, but not in excess leading to inflammation in the tissue.

4.3 | BCAA oxidation is downregulated in obesity

Adipose tissue is one of the main sites of mitochondrial BCAA catabolism.[89] BCAAs function as nutrient signals of amino acids, total food intake, and energy balance.[90,91] They regulate insulin secretion, protein synthesis, and protein breakdown in adipose, liver, and muscle tissues.[90,91] Already in the 1970s, adipose tissue was suggested to be important in the conversion of excess BCAA into fat[92]; the ability of which was confirmed only in 2010.[89]

In insulin resistance and in obesity, downregulation of BCAA oxidation enzymes and transcription of genes involved in BCAA oxidation in adipose tissue has been reported several times (Figure 2),[61,62,91,93-95] as have elevated levels of BCAAs in the blood stream.[61,96] The BCAA levels in circulation rise with decreased oxidation.[89] Increased plasma BCAA levels are associated with type 2 diabetes,[97] insulin resistance,[98] metabolic syndrome and cardiovascular diseases.[99,100] In contrast, BCAA-rich diets in mice on high-fat diet resulted in improved glucose metabolism and lower body weight compared with controls on high-fat diet without BCAA supplement.[101] The study, however, did not measure serum BCAA levels. Interestingly, elevated plasma levels of BCAA but improved insulin sensitivity and increased energy expenditure were observed in all-tissue BCATm-knockout mice.[102] Increased levels of BCAAs in circulation have been suggested to be contributors to the development of insulin resistance through the accumulation of mitochondriotoxic metabolites, which promote pancreatic β-cell dysfunction, apoptosis, and stress signaling.[90] Insulin resistance has indeed been shown to result in increased protein degradation in tissues,[90] leading to increased levels of amino acids and BCAAs in the circulation. Furthermore, persistent activation of the mammalian target of rapamycin complex 1 (mTORC1) by BCAAs appears to lead to uncoupling of the insulin receptor from the insulin signalling mediator (IRS-1) and to further insulin resistance.[90] It is unknown if the same mechanisms are involved in adipose tissue.

4.4 | Glucose oxidation is reduced in obesity

In high-fat–fed mice, metabolites related to glucose oxidation in the mitochondrial matrix are decreased, with a nearly 50% reduction in the levels of 1,5-anhydroglycitol (1,5-AG), which is a marker of short-term glycaemic control in plasma.[103] The levels of glucose-6-phosphate of glycolysis were decreased, and the expression of Pdk4, an inhibitor of mitochondrial pyruvate dehydrogenase activity, was diminished, suggesting aberrant glucose oxidation with high-fat diet.[103]

Mitochondrial glucose oxidation in adipose tissue is studied less in humans, and there is not yet direct evidence on decreased oxidation or increased glycolytic activity in human obesity. Comparisons between male and female subjects have shown that preadipocytes, but not mature adipocytes from female subjects, show higher mitochondrial to glycolytic activity in normoglycaemic conditions.[104] When challenged with extra glucose and insulin, preadipocytes from female donors reduce their ATP-linked mitochondrial respiration, possibly as a marker of greater insulin sensitivity, with both male and female preadipocytes increasing their glycolytic activity.[104] This might indicate changes in oxidative versus glycolytic activity with excess energy; however, more studies are needed to draw conclusions on the subject.

4.5 | Mitochondrial respiration and adipose tissue oxygen levels

Mitochondrial energy metabolism in the cell is oxygen dependent. Adipose tissue was previously suggested to be oxygen deprived in obesity, as the adipocytes enlarge beyond the oxygen diffusion limit[105] of approximately 100 μm. Hypoxia was considered as one of the inducers of inflammation, because preadipocytes obtained from individuals with obesity cultured in hypoxic conditions secreted more inflammatory markers.[106] In rodent models of obesity, rapid weight gain is accompanied with increased expression of genes related to hypoxia, lower partial oxygen pressure, and an increase in hypoxic areas in adipose tissue.[107,108] Also, decreasing adipocyte oxygen consumption and hypoxia by Ant2 knockdown in obese mice has led to decreased inflammation and improved glucose tolerance.[109] In human obesity, oxygen pressure in adipose tissue has been reported as reduced in individuals with obesity versus lean individuals.[110] However, recent new studies have questioned the view of hypoxia in adipose tissue in obesity. A study by Goossens et al has found increased oxygen pressure in adipose tissue of individuals with obesity.[111] The latter study was matched for age, gender, presence of type II diabetes, and ethnicity, and the results were later replicated in a weight-loss study by the same group, where 5-week very low–calorie diet in individuals with obesity decreased adipose tissue oxygen pressure.[112] Also, no significant differences have been found between persons with obesity and lean persons in relation to secretion of the adipose

tissue hypoxic markers lactate and pyruvate.[113] Based on these results, it has been suggested that the oxygen consumption of adipose tissue is reduced in obesity, resulting in higher adipose tissue partial oxygen pressure despite the lower blood supply.[114] As mitochondrial oxidative metabolism is an important consumer of cellular oxygen, these studies can be considered as indirect evidence for low mitochondrial oxidative metabolism in adipose tissue in obesity.

4.6 | Lipogenesis and lipolysis may be impaired in obesity

Active mitochondria are needed in both lipogenesis and lipolysis. Mitochondria are essential in generating the intermediary metabolites needed for *de novo* fatty acid synthesis[3] such as TCA-cycle–derived acetyl-CoA for fatty acid synthesis and esterification into triglycerides with glycerol-3-phosphate.[115] In mouse studies, the rate of lipogenesis decreases with interventions that reduce mitochondrial ATP generation.[116,117] Conversely, the amount of mtDNA shows a strong positive correlation with the rate of lipogenesis in human adipocytes.[65] Thus, mitochondrial dysfunction may be connected to impaired lipogenesis.

During fasting, adipose tissue releases FFA by lipolysis via the function of hormone-sensitive lipase (HSL)[118] and adipose triglyceride lipase (ATGL).[119] Lipolysis and mitochondrial ATP syntheses were first shown to be coupled in 1975, when inhibitors of the electron transport chain shut down catecholamine-induced lipolysis.[120]

During exercise or fasting, persons with obesity appear to have a lower capacity for lipolysis than lean persons, while in the fed state, lipolysis is insufficiently suppressed due to the insulin resistance of the adipose tissue, leading to hypertriglyceridemia.[121] Hypertriglyceridemia has a major role in the aetiology of insulin resistance.[5,122] Nevertheless, mice with an ATGL and HSL full-body knockout exhibit increased fat mass and lipid accumulation in insulin-sensitive tissues, however with inconsistent results on the insulin sensitivity of the animals.[123] Moreover, the levels of adipose tissue ATGL and HSL are shown to be decreased in insulin-resistant individuals with obesity.[124] In human multipotent adipose stem cell (hMADS) adipocytes, a knockdown or pharmacological inhibition of ATGL and HSL increased lipid accumulation and insulin resistance and leads to reduced mitochondrial oxygen consumption and impaired PPARα signaling.[125] The authors of the human and hMADS studies suggest HSL and ATGL deficiency as a compensatory mechanism, which tries to decrease lipid flux into the circulation while simultaneously impairing adipocyte function. Interestingly, inefficient lipolysis has recently been linked to future weight gain and suggested to be a marker for identifying risk individuals for the prevention of obesity.[126]

4.7 | Adipokine secretion is altered in obesity

Adipocytes and adipose tissue matrix cells secrete a variety of adipocytokines. These adipokines regulate energy intake and expenditure, body weight, glucose and lipid metabolism, insulin sensitivity and inflammation,[127,128] generation of preadipocytes,[129] and the migration of the cells in adipose tissue,[130] acting as hormones, or in an autocrine or paracrine fashion within adipose tissue.

Leptin and adiponectin are the main adipokines that are exclusively secreted by adipocytes. Leptin decreases food intake and energy consumption by acting on the hypothalamus and target tissues,[131] increases insulin sensitivity,[120] stimulates lipolysis by increasing cAMP concentrations in the cell,[132] and can activate inflammatory cytokine secretion.[133] However, apart from increasing cAMP concentrations, direct data on the relationship between leptin and mitochondria in the literature are sparse and still remain to be studied.

Adiponectin stimulates fatty acid oxidation, improves glucose metabolism and insulin sensitivity, and decreases plasma FFA levels in adipose tissue[134] by inhibiting lipolysis.[135] Adiponectin is also an anti-inflammatory molecule.[136] A decrease or an increase in mitochondrial biogenesis directly inhibits or enhances adiponectin secretion and synthesis in adipocytes.[137] This is evidenced also by inhibition of the electron transport chain or deletion of mitochondrial transcription factor A, which both lead to reduced adiponectin secretion.[138] Furthermore, hypertrophied adipocytes with diminished mitochondrial capacity have reduced synthesis of adiponectin.[139] In adipose tissue, adiponectin has been shown to block the mitochondrial apoptosis pathway by activating AMPK signalling and inhibiting ER stress-induced apoptosis.[140] These studies introduce adipocyte mitochondrial function as a potential target to restore adiponectin secretion and levels in obesity and related metabolic disorders.

4.8 | Mitochondria in adipogenesis

The renewal and maintenance of adipose tissue are achieved by preadipocytes. These cells are derived from adipose tissue stem cells,[141,142] which reside in adipose tissue vascular stroma.[142,143] Adipogenesis consists of two main steps: commitment of a pluripotent progenitor population to a preadipocyte lineage and terminal differentiation, in which the preadipocytes differentiate into mature functional adipocytes. Impairments in adipogenesis have been suggested to lead to accumulation of ectopic fat and insulin resistance.[2]

Mitochondria are essential in adipocyte differentiation and adipogenesis.[3] Mitochondrial biogenesis and adipogenesis are intertwined processes tightly coordinated by the same transcription factors. PPARγ,[144] C/EBPα,[144] CREB,[3,145] oestrogen-related receptor α (ERRα),[146] and PGC-1α are all major regulators of both adipogenesis and mitochondrial biogenesis[147,148] of which specifically PPARγ is a powerful inducer of adipogenesis. Mitochondrial biogenesis is thought to be under the control of adipogenic gene expression.[3,148] Various mitochondrial modifications take place during adipogenesis,[149] and mitochondria provide the essential substrates necessary for lipogenesis during adipogenesis.[115] A 20- to 30-fold increase in the amount of mitochondrial proteins has been recorded during adipocyte differentiation,[149] and the oxygen consumption of the preadipocytes increases in parallel to increased mitochondrial biogenesis.[149] The link between adipogenesis and mitochondrial biogenesis is reinforced also by the

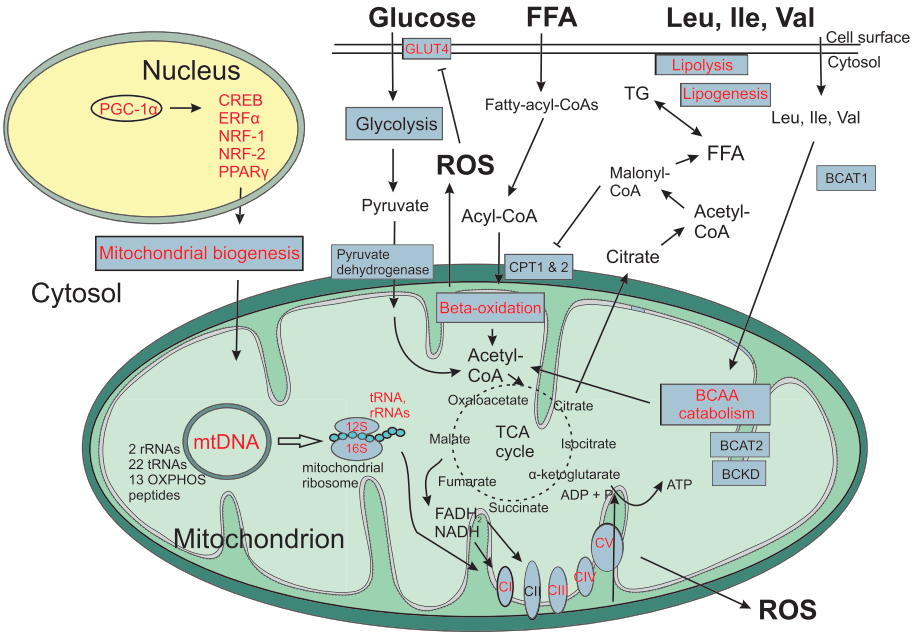

FIGURE 2 Mitochondrial dysfunction in adipose tissue in obesity. Steps leading to mitochondrial dysfunction: Downregulation of the nuclear transcription factors (red text) results into downregulation of mitochondrial biogenesis (in red) and adipogenesis with reduced mtDNA amount (red), reduced mitochondrial ribosomal transcripts (red), reduced OXPHOS transcripts and protein subunit levels (red). Reduction in the GLUT4 translocation (red) to adipocyte cell membrane reduces glucose uptake into adipose tissue and thus the levels of pyruvate available for TCA. Excess FFAs (in bold text) impair beta-oxidation (in red), with the production of excess ROS (bold) and possible intermediary oxidative metabolites. These further derange cell functions. Changes in lipogenesis and lipolysis (in red) take place, favouring fat storage; however, with also impairments in lipolysis. BCAA catabolism (in red) is downregulated yielding less Acetyl-CoA available for TCA. Mark explanations: text in red, downregulated in obesity; an arrow, induction; a T-line, inhibition; bolded text, increased in obesity

fact that the PPARγ agonist rosiglitazone induces alterations in mitochondrial morphology and density.[57]

Interestingly, BCAA catabolism and utilization of BCAA-derived carbon skeletons (through the TCA cycle) for fatty acid synthesis also increase during the differentiation,[150] potentially fuelling the process.[151] Mitochondrial metabolism of BCAAs also stimulates increase in fat cell mass, adipose tissue lipid uptake, and differentiation by activating mTORC and thus PPARγ signaling.[90,152]

In animal studies, pharmacologically inhibiting complex I or the ATP synthase inhibits the proliferation[153] and differentiation of preadipocytes in mice.[154] In mouse 3T3-L1 preadipocytes, mitochondrial dysfunction induced by a complex III inhibitor leads to abnormal triglyceride accumulation, reduced expression of adipogenic markers, and to impaired differentiation.[145] In rat preadipocytes, inhibition of complex I resulted in inhibition of preadipocyte differentiation with reduced ATP synthesis and downregulated gene expression of fatty-acid synthase, LPL, PPARγ, C/EBPα, and SREBP-1c.[155]

Adipogenesis and the enlargement of adipose tissue are seen as a crucial mechanism that protects other tissues from triglyceride accumulation, insulin resistance, and metabolic dysfunction.

4.9 | Mitochondria in adipocyte hypertrophy and hyperplasia in obesity

Adipose tissue enlarges by adipocyte hypertrophy (increase in cell size), hyperplasia (increase in cell number), or both (Figure 3). Mitochondria regulated lipogenesis and adipogenesis and thus may affect the hypertrophy versus hyperplasia of the tissue. The number of adipocytes in adipose tissue is determined by the differentiating preadipocytes and the recruitment of mesenchymal stem cells for differentiation.[156] In general, cellular hypertrophy is associated with insulin resistance,[157-159] hepatic steatosis, dyslipidemia,[160] elevated levels of circulating inflammatory markers,[161] increased inflammatory gene expression,[162] and increased number of macrophages in adipose tissue.[163,164] Hyperplasia in turn seems to preserve insulin sensitivity[158,165] and a favourable secretion of signalling molecules in adipose tissue.[163]

Adipocyte renewal and turnover—10% of the cells each year—occurs throughout life, although the number of adipocytes in an individual appears to be stable and determined early in adulthood.[166] Adipocyte number does not differ between co-twins with obesity compared with their lean MZ co-twins, suggesting a strong genetic control of this measurement.[167] There is no significant increase in adipocyte number in short-term weight increase[168] nor decrease following long-term weight loss.[166]

In individuals with obesity[169] and individuals with type 2 diabetes,[170] reduced generation of preadipocytes has been proposed, indicating defects in adipogenesis. In contrast, metabolically normal individuals with obesity have elevated levels of proadipogenic factors and lipid droplet proteins compared with metabolically compromised individuals.[171] Adipose stem cells isolated from human subjects with obesity show reduced mitochondrial content, ability to proliferate,

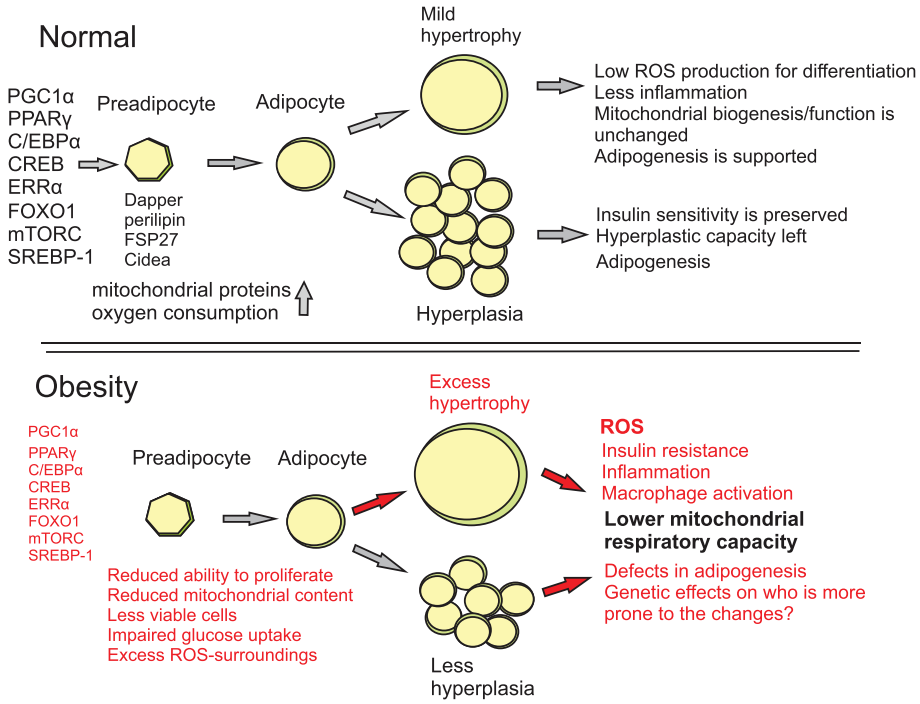

FIGURE 3 Adipogenesis, adipocyte hypertrophy and hyperplasia. The capacity of adipose tissue to enlarge is determined by the number of preadipocytes in the tissue and the capacity of preadipocytes to differentiate into adipocytes. Adipose tissue can enlarge by hypertrophy or hyperplasia. In normal state (above the line; black text, black arrows), nuclear transcripts induce preadipocyte recruitment and differentiation into adipocytes. Mitochondrial oxygen consumption and the amount of mitochondrial proteins increases. With increased fat mass, both hyperplasia and mild hypertrophy occur, preserving insulin sensitivity and mitochondrial function in the tissue. In obesity, excess FFAs stress the capacity of adipose tissue and its mitochondria. Nuclear transcripts inducing the differentiation of preadipocytes and mitochondrial function are downregulated (red text), leading to impaired differentiation and less mitochondria in the tissue (red). This in turn leads to less hyperplasia and increased hypertrophy of the cells (red arrows) leading to metabolic problems (red). However, some individuals with obesity appear to respond to increased FFA load more like in the normal state (black, above the line), with increased hyperplasia rather than only hypertrophy, avoiding part of the detrimental effects of fat accumulation. Mark explanations: red text, downregulated in obesity; black text, normal function; red arrows, metabolically unwanted changes; black arrows, normal function

and telomerase length and activity, and adipose stem cells in culture are less viable and more senescent than stem cells from lean persons.[172]

Small adipocytes are generally regarded as metabolically healthier. Their proportion is increased for example in lean co-twins of obesity-discordant twins,[167] and smaller adipocytes have been reported in omental adipose tissue of individuals with morbid obesity but with preserved insulin sensitivity[173] and in individuals characterized as "metabolically healthy" individuals with obesity,[174] without features of metabolic syndrome.[175] In contrast, an expanded population of small adipocytes and decreased expression of differentiation markers in the insulin-resistant versus insulin-sensitive individuals with obesity has been discovered, indicating potential defects in adipogenesis.[176] Insulin stimulates adipocyte hypertrophy,[177] but less hyperplasia.[178] Thus, the authors proposed that insulin resistance may hinder the hypertrophic effects of insulin with an impaired adipocyte differentiation towards larger mature cells.[176] However, based on the different studies, the insulin-sensitive persons with obesity, with a better adipocyte differentiation capacity, may be protected from the adverse effects of ectopic fat accumulation.

Arner et al have shown that subjects with a larger adipocyte volume than predicted for a given body fat mass (a hypertrophic adiposity phenotype) have lower rates of adipogenesis.[179] In turn, high generation rates of adipocytes associate with a hyperplastic phenotype.[179] After bariatric surgery, subjects with the hypertrophic obesity phenotype[180] had the largest reductions in adipocyte size and gained most in insulin sensitivity.[159] Interestingly, the adipocyte morphology in these latter studies was not associated with the amount of weight loss but rather to its metabolic outcomes indicating that the adipogenic capacity (regulated by mitochondria) of adipose tissue distinguishes the metabolic outcomes of obesity and of weight loss. However, both capacities—the hypertrophic and hyperplastic—seem to be needed for metabolic well-being.

We have previously associated large adipocyte volume to downregulation of mitochondria-related pathways in adipose tissue and these to the deterioration of metabolic profile in obesity.[167] The "metabolically unhealthy" twins with increased liver fat content compared with their leaner co-twins had hypertrophied adipocytes and less hyperplasia in their subcutaneous adipose tissue, with downregulation of mitochondrial oxidative pathways.[94] Our studies propose that some individuals may be able to respond to long-term energy excess with hyperplasia, while others predominantly react by hypertrophy, leading to differences in mitochondrial metabolism and the metabolic outcomes of obesity. A study by Yin et al in 2014 found reduced

mitochondrial respiratory capacity in individuals with obesity compared with lean individuals, independent of adipocyte size.[71] In 2015, Fisher et al also suggested that all adipocytes from an individual with obesity or from a lean individual have the same oxidative profile, independent of cell size, with obesity leading to impaired mitochondrial respiration in the cells.[75] These findings support the idea that obesity leads to deterioration of adipose tissue hyperplastic capacity and mitochondrial function; the exact relationship of which still requires further study.

4.10 | Visceral vs subcutaneous AT mitochondria

The studies on mitochondria in humans are largely concentrated on findings in subcutaneous adipose tissue, probably due to its easier accessibility for studies.

The visceral adipocytes in normal-weight rats contain more mitochondria than subcutaneous adipocytes.[181] In mice, subcutaneous adipocytes in turn seem to have a higher respiratory capacity than visceral adipocytes.[67] A high-fat diet in mice decreased visceral adipocyte mitochondrial capacity more than that of subcutaneous adipocytes, with the development of glucose intolerance.[67] In human studies, the mtDNA content in visceral adipose tissue was reduced in patients with diabetes but increased in patients with obesity compared with non-obese controls.[72] Human subjects with obesity undergoing bariatric surgery had twice the concentration of mitochondria per mass of tissue in visceral versus subcutaneous fat, but visceral fat had smaller cells to make up the same mass.[182] Respiration rates were higher in visceral than subcutaneous adipose tissue, but when normalized with mtDNA content, visceral adipose tissue had lower respiration per mitochondria and per adipocyte than subcutaneous adipose tissue.[182]

4.11 | Mitochondrial oxidative metabolism and insulin sensitivity

A link between adipocyte mitochondrial oxidative capacity and whole-body insulin sensitivity has been suggested by both rodent and human studies.[183] Insulin-sensitive and insulin-resistant patients with obesity appear to differ in adipose tissue oxidative stress levels and in the expression of genes related to mitochondrial function, SIRT1/Nampt activity,[111,173,184] and adipogenic capacity.[94] In obesity-discordant twins, the "metabolically unhealthy" group presented with reduced expression of mitochondria-related pathways and reduced mtDNA amount in subcutaneous adipose tissue with increased inflammation and insulin resistance.[94] These studies suggest an association between preserved mitochondrial function and insulin sensitivity.

More mechanistic studies in rodents reveal that excess glucose and fatty acids seem to lead to mitochondrial dysfunction. Mouse preadipocytes exposed to high glucose and fatty acids exhibit decreased mitochondrial size, decreased mitochondrial membrane potential, and downregulation of the master mitochondrial regulator PGC-1α.[185] Impaired glucose homeostasis, decreased PGC-1α expression, and reduced mtDNA content in response to high-fat feeding have also been demonstrated in rats.[186] High concentration of fatty acids in 3T3-L1 mouse adipocytes results in insulin resistance and in decreased levels of FoxO1, an inducer of adipogenesis and mitochondrial biogenesis.[54]

The mechanism linking mitochondrial dysfunction to the development of insulin resistance may involve the production of ROS. A high-fat diet in mice increased mitochondrial ROS, which were shown to contribute to insulin resistance in adipose tissue,[187] because genetic upregulation of the antioxidant enzyme MCAT was sufficient to reduce ROS damage and to preserve insulin signaling.[187] In another mouse study, high-fat feeding resulted in increased ROS and mitochondrial oxygen consumption rate and subsequently insulin resistance, which later led to a reduction in mitochondrial biogenesis.[188] Earlier studies have also linked the increase in oxidative radicals O_2 superoxide and H_2O_2 to insulin resistance,[187,189,190] and a long-term high-fat diet is associated with increased oxidative stress markers in humans and mice.[191] A recent study on 3T3-L1 adipocytes and adipose tissue revealed a mechanism for the latter relationship, where elevated mitochondrial oxidants rapidly impair insulin-regulated GLUT4 translocation and lead to insulin resistance in tissue.[192] Furthermore, another study in 3T3-L1 preadipocytes demonstrated that reduction in mtDNA levels and respiratory chain activity actually enhanced insulin signalling but nevertheless caused impaired insulin responsiveness by decreasing GLUT4 translocation on the cell surface.[193]

Taken together, these studies link mitochondrial oxidative activity and particularly ROS generated in the electron transport chain to insulin sensitivity. Excess fatty acids and glucose are the potential initiators of mitochondrial problems. This idea is in line with studies where lipid metabolites such as ceramide and diacylglyceroles (DAG) cause insulin resistance and impair mitochondrial function by activating serine kinases.[194-196] Furthermore, interventions that have compromised mitochondrial function by genetic[193] or pharmacologic[197] mechanisms have resulted in insulin resistance.

The connection between mitochondrial respiratory activity to insulin resistance has not been replicable in all studies. A murine study found reduced mitochondrial parameters in both obese and diabetic mice[60] and respiratory capacity in subcutaneous and visceral fat was reduced in obese mice, independent of insulin resistance.[58] No connection between mitochondrial biogenesis and glucose homeostasis was observed in a study on mouse adipocytes[198] and no association between type 2 diabetes and the activity of the mitochondrial OXPHOS complexes in human visceral adipose tissue.[199]

4.12 | Mitochondrial oxidative metabolism and inflammation

Inflammation is closely linked to adipose tissue dysfunction in obesity. Although downregulation of mitochondrial capacity and increased inflammation frequently co-occur in adipose tissue in obesity,[62,94,95,200,201] the order of the events is not clear. Mitochondrial dysfunction can be both the cause and the consequence of

inflammation. However, many studies suggest that mitochondrial dysfunction precedes inflammation in adipose tissue.

A study with diet-induced obese mice showed increased activity of pathways that favour fat storage and prevent lipid oxidation, with an early decrease in citrate synthase activity and expression of Pgc1a, with these changes preceding the inflammatory cell infiltration and the decrease in mitochondrial abundance.[103] FFA and glucose in high quantities directly stimulate mitochondrial dysfunction[185] and have led to high levels of TNFα, ER stress, and increased ROS[202,203] in mouse 3T3-L1 preadipocytes. The mechanisms by which mitochondrial dysfunction causes inflammation could include increased ROS production, which together with intermediary metabolic compounds[204,205] predisposes to cell death and consequently to increased immune cell infiltration.[206] Accumulation of intermediary metabolic compounds—protein carbonyls, lipid peroxidation products, and malondialdehyde—together with increased ROS production and mitochondrial oxidative stress has been observed in adipose tissue of subjects with obesity, with obesity and diabetes and non-obese subjects with diabetes compared with lean subjects.[205] Expanding large adipocytes (with potential mitochondrial dysfunction) express inflammatory markers[207,208] and release more chemokines[207,208] and FFA, which are able to recruit macrophages into adipose tissue and activate inflammatory pathways.[209] Furthermore, mitochondria synthesize phospholipids, which are responsible for the fluidity of the adipocyte cell membrane,[210] and membrane lipid modifications in humans have been shown to recruit inflammatory cells into adipose tissue.[211]

Based on these studies, obesity may disrupt mitochondrial balance, leading to increased oxidative stress and increased inflammation in the adipose tissue. On the other hand, inflammation may lead to further deterioration of mitochondria-related metabolism in the cells, as evidenced by studies where preadipocytes obtained from mice[212] or preadipocytes isolated from individuals with obesity[213] have been treated with proinflammatory TNF-α. However, more studies are still needed on the exact causal relationship of mitochondrial dysfunction and inflammation in adipose tissue in obesity (Figure 4).

4.13 | Methods to study mitochondria in adipose tissue

There are many methods to study mitochondria. In this paragraph and in Table 1, we briefly summarize the main methods that have been used in white adipose tissue. On global level, the expression of genes related to mitochondria has been studied via transcriptomics from

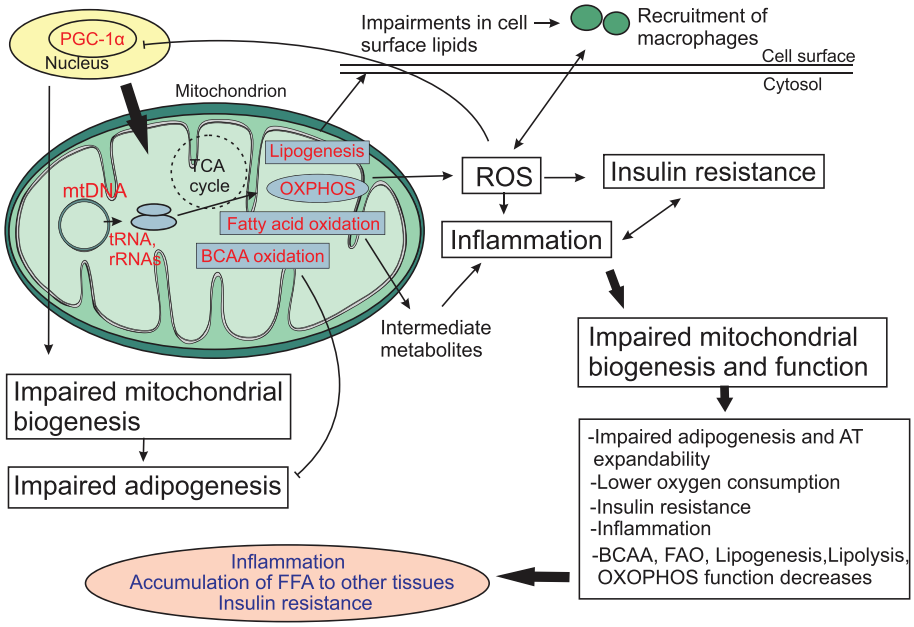

FIGURE 4 Mitochondrial dysfunction in relation to whole-body metabolism. In obesity, mitochondrial biogenesis and function with lipogenesis and lipolysis, oxidative phosphorylation, FFA oxidation and BCAA oxidation (main mitochondrial pathways, marked in red) appear to be impaired. Impaired mitochondrial biogenesis (an arrow from nucleus passing through mitochondria, presenting downregulation of mtDNA amount, mitochondrial mtRNAs and rRNAs, PGC-1α, OXPHOS subunits) is connected with impaired adipogenesis. Downregulation of BCAA oxidation also blocks the induction of adipogenesis by BCAA metabolites (a T-line BCAA-adipogenesis). Impaired OXPHOS produces excess ROS, leading to increased inflammation and insulin resistance in adipose tissue. ROS also activate the adipocytes to recruit macrophages into the tissue. Inflammation further impairs mitochondrial biogenesis. Changes in the generation of phospholipids for cell surface reduce the fluidity of the cell membrane and make the tissue more prone to inflammatory cell infiltration. These changes lead to impaired mitochondrial biogenesis and function (bold arrow). Mark explanations: red text, downregulated mitochondria-related transcripts or mitochondrial components in obesity; an arrow, induction; a T-line, inhibition; bolded arrows, main pathways leading to metabolic derangements. As a result, impaired adipogenesis, lower oxygen consumption of adipose tissue, increased insulin resistance and inflammation, and reduced oxidative and metabolic functions of adipose tissue are observed. These changes further connect with the accumulation of FFA to ectopic sites and internal organs, increased inflammation and insulin resistance in the whole-body level (oval, orange background)

TABLE 1 Methods to study mitochondria

1. DNA, RNA, gene expression (nucleus), proteome			**Conclusions**
1. RNA (gene expression)—mitochondria-related nuclear transcripts	Affymetrix, other microarrays RT q-PCR, RNA-seq	(Pietiläinen, PlosMed, 2008) (Heinonen, Buzkova, Diabetes 2015) (Rong JX, Diabetes 2007) (Wilson-Fritch, J Clin Invest 2004) (Vernochet, FASEB 2014) (Lee, Cell Rep 2016) (Sutherland LM, Am J Physiol Endocrinol Metab. 2008) (Bogacka I, Diabetes 2005)	Mitochondria-related nuclear transcriptional signature is downregulated in obesity Downregulation of main mitochondrial regulatory transcripts
2. Methylation studies	Infinium Human-Methylation450K BeadChip	(Heinonen, Buzkova, Diabetes 2015) (Ejarque, Int J Obes 2018)	Obesity leads to changes in methylation Preadipocytes from obese donors maintain obesity-conditioned state in culture. Inflammation, adipogenesis most affected pathways.
3. Proteome studies	Mass spectrometry	(Wilson-Fritch, Mol Cell Biol 2003) (Xie, Obesity 2016) (Lindinger PW, Proteomics 2015) (Gomez-Serrano Sci Rep 2016)	Reduced levels of mitochondria-related proteins in obesity Reduced levels of mitochondria-related proteins in diabetes (without obesity)
2. Mitochondrial mtDNA, mtRNAs, protein subunits			**Conclusions**
1. mtDNA copy number	DNA extraction from tissue, qPCR to determine the mtDNA level relative to nuclear DNA	(Heinonen Buzkova, Diabetes 2015) (Pietiläinen et al, PlosMed 2008) (Rong JX, Diabetes 2007) (Ande S, Diabetes 2014) (Lee, Cell rep 2016) (Bogacka I, Diabetes 2005) (Schöttl T, Endocrinology 2015) (Sutherland LM, Am J Physiol Endocrinol Metab. 2008) (Bogacka I, J Clin Endocrinol Metab. 2005) (Kaaman M, Diabeteologia 2007) (Yehuda-Shnaidman, Diabetes 2010) (Yin, J Clin Endocrinol Metab 2014) (Lindinger, Obes Surg 2010)	Downregulation of mtDNA copy-number in obesity No difference in mtDNA content between individuals with obesity and lean persons Increased mtDNA content in obesity
2. mtDNA transcripts	Rt-qPCR, primers designed for mitochondrial mRNAs	(Heinonen Buzkova, Diabetes 2015) (Heinonen et al, Diabetes 2016)	Downregulated in obesity
3. OXPHOS subunit levels	Western Blot: Antibodies directed against OXPHOS complex subunits	(Heinonen Buzkova, Diabetes 2015) (Heinonen et al, Diabetologia 2016) (Fischer, Am J Phys Endo Metab 2015) (Vernochet, FASEB 2014, in mice) (Schöttl T, Mol Metab 2015, in mice) (Ande S, Diabetes 2014, in mice)	Lower levels of mitochondria-related OXPHOS subunits in obesity

(Continues)

TABLE 1 (Continued)

	OXPHOS and other proteins	(Sutherland LM, Am J Physiol Endocrinol Metab. 2008) (Lee, Cell rep 2014)	
4. Isolation of mitochondria from the tissue		(Chattopadhyay et al, Metabolism 2011) (Fischer, Am J Phys Endo Metab 2015) (Yin, J Clin Endocrinol Metab 2014) (Vernochet, Cell metab 2012) (Yang, Diabetes 2016) (Vernochet, FASEB 2014) (Schöttl T, Endocrinology 2015) (Schöttl T, Mol Metab 2015)	Purified mitochondria used for Western blotting, respiration analyses
5. Mitochondrial microscopy for mitochondrial structure and dynamics	MitoTracker staining, fluorescence microscopy MitoTracker green for mitochondrial content + network Mitosox red for redox state Electron microscopy of fixed AT samples	(Wilson-Fritch, J Clin Invest 2004) (Perez, Plos One 2015) (Ande S, Diabetes 2014)	Rosiglitazone increases mitochondrial density Higher mitochondrial content in ASC, produce more ROS Increased mitochondrial content in transgenic mice
3. Functional measurements			**Conclusions**
1. Respiration measurements	Oxygraph-2k; Oroboros high resolution respirometry (isolated mitochondria, isolated adipocytes)	(Yin et al, J Clin Endocrinol Metab 2014), human adipocytes -isolated mitochondria (Schöttl T, Endocrinology 2015, mice study) -adipocytes	Lower mitochondrial oxygen consumption rates (OCR) with adipocytes from obese donors, independent of cell size Lower OCR respiration rates with adipocytes in visceral AT compared to subcutaneous tissue Lower OXPHOS capacity in obesity Limited mitochondrial OXPHOS capacity in obesity Mitochondrial function reduced in obese-derived preadipocytes
	Seahorse XF-96 extracellular flux analyser of ASc or isolated mitochondria, OCR oxygen consumption, ECAR extracellular acidification Spectrophotometric analysis of isolated respiratory chain complex activities (incl. citrate synthase	(Schöttl T, Mol Metab 2015), mice -adipocytes (Schöttl T, Mol Metab 2015) (Schöttl T, Endocrinology 2015) (Perez, Plos One 2015) -cultured preadipocytes (Vernochet, Faseb 2014)	Activities reduced in transgenic mice (TFAM knockout) with lipodystrophy and mitochondrial dysfunction
Enzymatic activity of OXPHOS		(Chattopadhyay et al, Metabolism 2011)	
2. Enzymatic activity of part of OXPHOS or metabolite levels related to mitochondria	Citrate synthase activity by spectrophotometer	(Rong JX, Diabetes 2007) (Yin, J Clin Endocrinol Metab 2014) (Yang Diabetes 2016) (Schöttl T Endocrinology 2015)	Overall conclusions: metabolite levels/enzyme activity activities reduced in obesity indicating reduced mitochondrial function Lower in obesity

(Continues)

TABLE 1 (Continued)

Inorganic phosphate utilization of mitochondria	(Schöttl T Mol Metab 2015)	Lower in AT of mice with genetic mitochondrial dysfunction
ATP content with Luminometry	(Chattopadhyay et al, Metabolism 2011)	Lower in obesity
Fumarate, succinate levels	(Yang, Diabetes 2016)	Loss of membrane potential with excess glucose and FFA
Mitochondrial membrane potential with fluorescence		Increased in obese mice
Mitochondrial ROS measurement	(Gao JC, Mol Cell Endocrinol 2010)	Reduced production in obese ASCs
Lactic acid levels from ASCs	(Gao JC, Mol Cell Endocrinol 2010) (Schöttl T Mol Metab 2015) (Perez Plos One 2015)	

Note. Table 1 presents methods used to study mitochondria in adipose tissue, with the different structures or functions of mitochondria listed on the left followed by analyses and the laboratory techniques, studies using these methods, as well as the common conclusions on each subject.

RNA isolated from adipose tissue or purified adipocytes.[57,60-62,81,186] Mitocarta, a public inventory of 1158 human and mouse genes that encode proteins with strong support of mitochondrial localization,[34] can help with finding the transcripts related to mitochondria. Similarly, proteomic studies by mass spectrometry determine the amount and distribution of mitochondria-related proteins of interest.[77-79] Isolation of DNA and the epigenetic studies has been used to determining the activity or silencing via methylation of certain mitochondria-related genes.[62,76]

More targeted analyses can be made from mitochondria isolated from the tissue.[71,74,75] The mtDNA copy number in mitochondria by qPCR may be determined by specifically targeted primers to evaluate the mitochondrial DNA amount relative to nuclear DNA.[61,62,65,67,186] As mitochondria may possess multiple copies of their genome, mtDNA mass does not equal the number of mitochondria in the tissue. The transcript levels of the mtDNA encoded genes can be studied by mt-RNA targeted quantitative RT-PCRprimers.[62,63] The levels of mitochondria-encoded protein subunits of the OXPHOS complexes by western blot may be determined.[58,62,63,75,81] Immunoblotting can be used to study the levels of also other specific nuclear-encoded mitochondria-related proteins.[58,69,81,186]

Mitochondrial staining by MitoTracker Green or other mitochondrial dyes for fluorescence microscopy determinations add to the knowledge on mitochondrial morphology and distribution[57,58,69,81] The ultrastructure of mitochondria have been further studied with transmission electron microscopy.[69]

Studies assessing mitochondrial function directly in adipose tissue or preadipocytes include respiration capacity measurements of preadipocyte cultures or isolated mitochondria by Seahorse extracellular flux analyzer[58,172] or respirometry analyses from isolated mitochondria or adipocytes by Oroboros O2K high-resolution respirometry.[58,67,71] Spectrophotometric analyses of isolated respiratory chain complex activities have also been used to assess OXPHOS activity.[74,81] Also, single enzyme activities in pathways other than OXPHOS, such as citrate synthase activity,[58,60,67,71] have been used as an indicator of mitochondrial activity.

Indirect ways of studying mitochondria include methods, where the oxygen consumption of the tissues is calculated through measuring blood flow in the tissue,[111,112] through the inorganic phosphate utilization of mitochondria[74] or other metabolites entering and formed in adipose tissue or cultured preadipocytes by spectrophotometric assays: ATP content,[80] fumarate, succinate,[185] or other mitochondria-related metabolites.

4.14 | Interventions to enhance mitochondrial biogenesis in AT

Enhancing mitochondrial amount or function in adipose tissue may help with addressing the metabolic complications of obesity.

Some pharmacological interventions that have already been tested to treat insulin resistance could increase mitochondrial biogenesis in obesity. PPARγ-agonist thiazolidinediones (TZDs) have

been used to treat patients with type 2 diabetes. These agents improve the insulin sensitivity of adipose and other tissues. TZDs appear to increase mitochondrial biogenesis, function, and content. They improve oxidative capacity in adipocytes in mice and upregulate mitochondria-associated genes, mtDNA amount, and PGC-1α.[57,149] In humans, evidence of this has also been shown in vitro[214] and in vivo, where treatment with pioglitazone improved mitochondrial biogenesis in patients with diabetes by stimulating the expression of genes important for fatty acid oxidation and PGC-1α and by increasing mitochondrial copy number.[66] However, rosiglitazone from the same TZD group caused cardiovascular problems, which led to its withdrawal from use.[215,216] In humans, treatment with vitamin D[217] reduced oxidative stress and improved insulin sensitivity. Vitamin E in mice activated PPARalpha, the mediator of mitochondrial fatty acid oxidation, and improved insulin sensitivity.[218] R-alpha-lipoic acid and acetyl-L-carnitine enhanced mitochondrial biogenesis and fatty-acid oxidation in cultured mouse adipocytes.[219] The same compounds have resulted in improvement in insulin sensitivity in human studies, although no relation to mitochondria was studied.[220,221] However, other human studies have failed to show the association between antioxidants and improved metabolism.[222,223] Chemical uncouplers that dissipate the energy produced by mitochondria as heat have led to weight loss[224] but also to uncontrolled thermogenesis with fatal increases in body temperature.[225] Use of chemical uncouplers has thus discontinued. In muscle studies, exercise[226,227] (with PGC-1α and VEGFA upregulation[228]) and caloric restriction in muscle and adipose tissue (AMPK and SIRT1 as the principal modulators[229,230]) appear to improve mitochondrial biogenesis. However, data from adipose tissue are still sparse. In mouse studies, resveratrol and other small-molecule compounds that activate SIRT1 have led to improvement in insulin sensitivity in adipose tissue, muscle, and liver[231,232] and increased the expression of mitochondria-related genes in liver and muscle tissue.[232]

Bariatric surgery has also emerged as a possibility to improve metabolic health in obesity. After surgery, insulin-resistant individuals exhibit increased levels of mitofilin (a regulator of mitochondrial membrane architecture), increased PGC-1α protein levels, and upregulation of mitochondrial superoxide dismutase.[233] In normoglycaemic individuals, mitofilin and PGC-1α were already high before bariatric surgery and decreased afterwards.[233] Bariatric surgery may thus enhance mitochondrial biogenesis, activity, and antioxidant defences in adipose tissue of insulin-resistant subjects with obesity, but more studies on this are still needed.

Obesity is a multisystemic and multifactorial disease. Personalized therapies will be needed to treat individuals with obesity with different metabolic status and to administer drugs locally.[234] Targeting mitochondrial metabolism may decrease insulin resistance and inflammation and enhance preadipocyte differentiation in adipose tissue with beneficial effects in the treatment of obesity. Targeted antioxidant supplements, mild and safe mitochondrial uncoupling agents with thiazolidinedione-type drugs, or both may be beneficial.

5 | CONCLUSIONS

Normal mitochondrial function has central effects on the health and function of adipose tissue. The downregulation of mitochondrial oxidative metabolism in obesity has been shown in many animal and human studies, involving both adipose tissue and adipocytes, with changes in gene expression as well as in the methylation pattern of the cells. Disruptions in adipocyte mitochondrial function and increased demand for FFA and glucose disposal in obesity lead to enhanced lipid deposition in the cell.[145] This may lead to enhanced stress, accumulation of intermediary metabolites, increased inflammation and ROS, and reduced mitochondrial biogenesis. This can further lead to reduction in adipogenesis, oxidative metabolism, and insulin sensitivity of the cells,[2,4] as well as to inflammation. If adipose tissue and its mitochondria are dysfunctional and the storage capacity of adipocytes is compromised, FFA spill over to ectopic sites in the liver, muscle, and pancreas causing dysfunction and insulin resistance.[235] Mitochondrial dysfunction also reduces the synthesis of adiponectin.[137-139] Adipose tissue dysfunction has been recognized as an important contributor to obesity-related disorders[5,6] and can increase the risk of developing insulin resistance and metabolic complications of obesity.[9,236] In the light of these findings, it seems plausible to suggest mitochondria as one of the underlying causes behind the metabolic complications that develop during obesity. (Figure 4)

Based on current knowledge, there is a widespread downregulation of adipose tissue mitochondria in obesity, although with some controversy on the causal relationships between low mitochondrial oxidative function with inflammation, insulin resistance, and deranged differentiation of cells. These aspects require more follow-up studies in humans. Downregulation of mitochondrial function or biogenesis in obesity has long-term consequences for the metabolism of adipose tissue and the whole body. (Figure 4)

Future research on understanding the exact pathways that lead to mitochondrial dysfunction in adipose tissue in obesity and revealing possible targets for pharmacological or nutritional interventions to enhance mitochondrial biogenesis or function in obesity is warranted. Distinguishing the individuals most prone to the metabolic problems of obesity and who would gain most of the interventions will be an important future target.[62,94] More mechanistical studies are needed on the relationship of mitochondrial function, inflammation, and insulin resistance to be able to answer this need.

Mitochondria in white adipose tissue may in future be pharmacologically modified to enhance their function and amount and provide metabolic benefits for people with obesity. Compounds already identified for this purpose include agonists for the PPARs and ERRs, SIRT1, TGR5, and AMPK.[237] However, these compounds only target one specific site in a complex network, and thus, compounds with a broader effect, targeting many dysfunctional sites, should be identified with computer-based methods.[237] These compounds can include ones that restore mitochondrial function and also those that are toxic to mitochondria and should be avoided.[237]

Specific diets to promote mitochondrial function may be studied. The types and amount of exercise to elicit best benefits for adipose

tissue mitochondria may be investigated.[238] Imitating interventions like bariatric surgery that seem to preserve a favourable metabolic profile in adipose tissue should be investigated further. Combining different strategies will be a key issue in combating a multifactorial disease like obesity.

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CONFLICT OF INTEREST

The manuscript has been accepted by all co-authors; all had access to the materials and data and hold the final responsibility for the submission of the article. We state that the study is original, has not been submitted for publication in other journals, and has not yet been published either whole or in part. The authors declare no conflicts of interest with this article.

AUTHOR CONTRIBUTIONS

SH wrote the manuscript. RJ, KHP, and AR participated in the writing and KHP, RJ, and AR in the revision of the work. KHP designed the research and supervised the work. KHP is the guarantor of this work and as such had full access to the data and takes responsibility for the integrity of the data.

ORCID

Sini Heinonen https://orcid.org/0000-0002-6342-0577

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